Effects of pH on uranium uptake and oxidative stress responses induced in *Arabidopsis thaliana*

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1. INTRODUCTION

Uranium (U) is a naturally occurring radionuclide and heavy metal, with a greater risk of chemical toxicity than radiological toxicity because of its low specific activity. U can be present in a wide variety of chemical species, which can be divided into three predominant species: uranyl cation, uranyl hydroxides and uranyl carbonates [1]. Important factors controlling the speciation are for example pH value, redox potential, ionic strength and availability of inorganic and organic ligands [2].

Exposure of plants to environmental stress conditions (eg. heavy metals) can lead to oxidative stress. Oxidative stress is the disturbance of the cellular redox status, caused by inhibition of the antioxidative defence system (enzymes and metabolites) and/or increased production of reactive oxygen species (ROS) [3]. However, ROS have a dual role as both toxic byproducts of aerobic metabolism and key regulators of growth, development and defence pathways [4].

It is already demonstrated that U can cause oxidative stress in *Arabidopsis thaliana* plants grown at pH 5.5 [5]. However, it is unknown how the different U-species present at different pHs determine the U-uptake and translocation within plants and affect the oxidative defence mechanisms of these plants. To evaluate the environmental impact of U-contamination, it is important to unravel these mechanisms under ecological relevant conditions.

The aim of this study is to analyze the biological effects induced in *Arabidopsis thaliana* exposed to U at different pHs. We aimed to analyze growth responses and the antioxidative defence system of the plants.

2. MATERIALS AND METHODS

Arabidopsis thaliana seeds (Columbia ecotype) were incubated in the dark for three days at 4°C on moist filter paper to synchronize germination. Seeds were sown on plugs from 1.5 mL polyethylene centrifuge tubes filled with 0.6% agar. The plugs were positioned in a PVC cover capable of holding 36 plugs. Next, the

cover was placed on a container filled with 1.35 L of a modified Hoagland solution with a pH of 5.5. Plants were grown in a growth chamber (Microclima 1000E, Snijders Scientific B.V.) under a 14 h photoperiod (photosynthetic photon flux density of 200 µmol m^{-2} s^{-1} at the leaf level), with day/night temperatures of 22°C/18°C and 65% relative humidity. After 18 days preculture, the pH of the nutrient solution was adjusted to different pHs ranging from 4.5 to 7.5 in steps of one unit. To retain the pH at a constant level, 500 µM MES (2-(N-morpholino)ethanesulfonic acid) and 500 µM TRIS (tris(hydroxymethyl)-aminomethane) were used. For each pH treatment, 25 µM ^{238}U was added to half of the plants, while the other half were considered the control plants. Uranium was added as $UO_2(NO_3)_2.6H_2O$ (Sigma) to the Hoagland nutrient solution. During the exposure time, a modified Hoagland solution was used with 1/80 phosphate solution [5]. After 3 days exposure, plants were harvested.

At harvest, root and shoot biomass were determined. Samples were taken to measure U-concentration in roots and shoots. The rest of the plants were snap-frozen in liquid nitrogen and stored at -80°C for the determination of lipid peroxidation, according to Dhinsda et al. [6] and to measure the activities of enzymes of the antioxidative defence mechanism, as was done before [5, 7].

Statistical analyses were performed using an ANOVA test [8] in SAS 9.2. The ANOVA test was carried out separately for leaves and roots. Mean values of treatments were compared using Tukey's multiple comparison test. Transformations were applied when necessary to approximate the assumptions of normality and same error variance. If the assumption of normality was not fulfilled, a non-parametric Wilcoxon rank sum test was carried out.

3 RESULTS AND DISCUSSION

After 3 days exposure of *Arabidopsis thaliana* seedlings to 25 µM U, the U-concentration in the roots was more than 100 times higher than in the leaves, indicating a small root-to-shoot transfer ranging from 0.0005 (pH 4.5) to 0.006 (pH 7.5). Plants grown at pH 7.5 have a U-concentration in the shoots twice as high compared to the other pHs (figure 1). In the roots, the highest U-concentration was found in plants exposed to pH 4.5. These results indicate that there is a different rate in U-uptake and translocation at the different pHs, with the highest uptake at pH 4.5 and the highest translocation at pH 7.5.

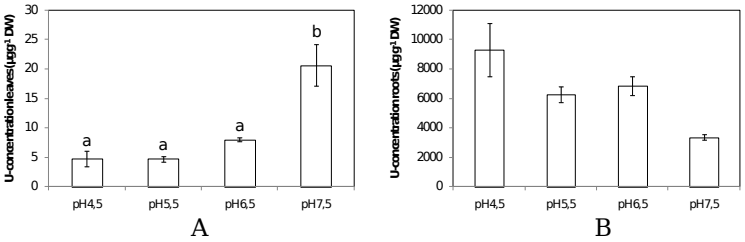

Figure 1. Uranium concentration (µg g^{-1} DW) in *Arabidopsis thaliana* leaves (A) and roots (B) exposed to 25 µM U for 3 days at different pHs (4.5, 5.5, 6.5 and 7.5). Values represent mean ± S.E. of at least 4 biological replicates. Data points with different letters are significantly different ($p<0.05$).

There is no effect of the pH on the biomass of control plants (without U). However, in the U-exposed plants, there is a decreased root and shoot biomass at pH 4.5 compared to pH 5.5. In the U-exposed leaves, there was an increased biomass at pH 6.5 and 7.5 compared to pH 5.5. By comparing the U-treated and

control plants for the same pH, there is only an effect in the leaves at pH 4.5 and pH 5.5 (figure 2).

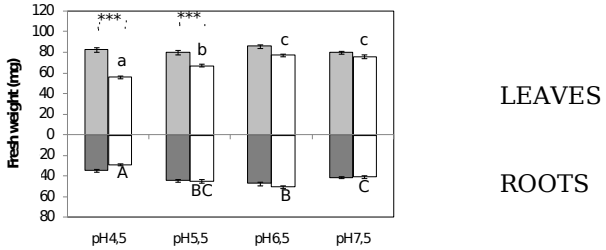

Figure 2. Fresh weight (mg) of *Arabidopsis thaliana* leaves (upper part of the graph) and roots (lower part of the graph) of the non-exposed plants (solid bars) and plants exposed to 25 µM U (striped bars) at different pHs (pH 4.5, 5.5, 6.5, 7.5). Values represent the mean ± S.E. of at least 109 biological replicates. Statistical analyses were done separately for leaves and roots. Data points with different letters are significantly different ($p<0.05$). *** indicates differences between control plants and U-exposed plants at the same pH ($p< 0.001$).

Lipid peroxidation is a measure for membrane damage and dysfunction and can be analyzed by measuring the TBA reactive compounds. In the roots, there was an increasing trend in the TBA concentration for the U-exposed roots compared to the non-exposed roots. In the U-exposed leaves at pH 4.5 there was a significant increase in TBA concentration compared to exposed plants at the other pHs used and also compared to the non-exposed leaves at pH 4.5 (figure 3). This effect is probably generated via root-to-shoot signaling due to the high U-concentration present in the roots at pH 4.5, as almost no U-translocation to the leaves was observed.

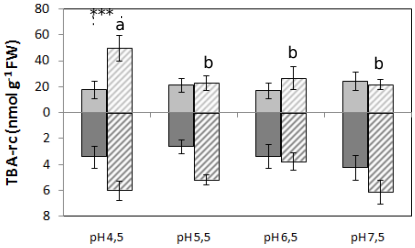

Figure 3. Level of lipid peroxidation, based on the amount of TBA reactive compounds, in *Arabidopsis thaliana* leaves (upper part of the graph) and roots (lower part of the graph) of the non-exposed plants (solid bars) and plants exposed to 25 µM U (striped bars) at different pHs (pH 4.5, 5.5, 6.5, 7.5). Values represent the mean ± S.E. of at least 3 biological replicates. Statistical analyses were done separately for leaves and roots Data points with different letters are significantly different ($p<0.05$). *** indicates differences between control plants and U-exposed plants at the same pH ($p<0.001$).

Enzymes of the antioxidative defence system were analyzed on protein level to evaluate the importance of the cellular redox balance in *Arabidopsis thaliana* plants exposed to U at different pHs. In the roots, there was an increased activity of guaiacol peroxidase (GPX) (all pHs) and glutathione reductase (GR) (pH 4.5). However, there was almost no increase in superoxide dismutase, catalase or ascorbate peroxidase activity. GPX plays an important role in cell wall lignification, a defence reaction that limits the entry of toxic metals into the roots [9]. GR is important in the recycling of reduced glutathione (GSH) from oxidized glutathione (GSSG). GSH plays an important role in the U-response: on the one hand, GSH is an important antioxidant. On the other hand, GSH is a

precursor of phytochelatines. Phytochelatines are heavy metal-binding peptides that are important in the detoxification of toxic heavy metals [10]. So it seems that in the roots, plants try to avoid the toxic effects of U by reducing the free cellular U.
In the leaves, however, there is an increased activity of SOD and CAT after U-exposure, which could indicate that antioxidative defence mechanisms were activated.

4. CONCLUSION

This study aimed to analyze effects on growth and the antioxidative defence system of *Arabidopsis thaliana* plants after U-exposure at different pHs. It seems that at pH 4.5 *Arabidopsis thaliana* plants are more sensitive to U than at higher pH. Although growth responses and lipid peroxidation were mainly observed in the leaves of U-exposed plants at pH 4.5, the U-concentration in those leaves was 4 times lower compared to pH 7.5. The high U-concentration in the roots of plants exposed at pH 4.5 can probably lead to a root-to-shoot signaling, which causes the observed effects in the leaves of those plants.
The high activity of GPX and GR in the roots seems to indicate that the plants try to complexate U in the roots. In contrast, in the leaves the antioxidative defence mechanism was activated. To further investigate these hypotheses, phytochelatines will be determined in the roots. The concentration of different metabolites of the antioxidative defence mechanism will also be analysed, which are important in redox homeostasis [11].

References

1. S. D. Ebbs, D. J. Brady, W. A. Norvell and L. V. Kochian, Pract. Periodical of Haz., Toxic, and Radioactive Waste Mgmt **5** (2001) 130-135.
2. G. Bernhard, Landbauforschung Völkenrode **55** (2005) 139-148.
3. T. Remans, K. Opdenakker, K. Smeets, D. Mathijsen, J. Vangronsveld and A. Cuypers, Functional Plant Biology **37** (2010) 532-544.
4. R. Mittler, S. Vanderauwera, M. Gollery and F. Van Breusegem, Trends in Plant Science **9** (2004) 490-498.
5. N. Vanhoudt, H. Vandenhove, K. Smeets, T. Remans, M. Van Hees, J. Wannijn, J. Vangronsveld and A. Cuypers, Plant Physiology and Biochemistry **46** (2008) 987-996.
6. R. S. Dhindsa, P. Plumbdhindsa and T. A. Thorpe, Journal of Experimental Botany **32** (1981) 93-101.
7. N. Vanhoudt, H. Vandenhove, N. Horemans, J. Wannijn, A. Bujanic, J. Vangronsveld and A. Cuypers, Plant Physiology and Biochemistry **48** (2010) 879-886.
8. J. Neter, M. H. Kutner, C. J. Nachtsheim and W. Wasserman, in *Applied linear statistical models*, 4th edn.(Boston, Massachusets, The McGraw-Hill Companies, 1996).
9. R. L. Ederli L, Ferranti F, Pasqualini S, Physiologia Plantarum **121** (2004) 66-74.
10. K. Hirata, N. Tsuji and K. Miyamoto, Journal of Bioscience and Bioengineering **100** (2005) 593-599.
11. G. Queval and G. Noctor, Analytical Biochemistry **363** (2007) 58-69.